

2-16-95

MRID No. 429024-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Oxine Copper.
Shaughnessey No. 024002.
2. **TEST MATERIAL:** 1) Quinolinate 98; oxine copper or copper 8-quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) ^{14}C -oxine copper; Lot No. 029F9233; specific activity of 92.9 $\mu\text{Ci}/\text{mg}$; 98.25% active ingredient.
3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Dunaliella tertiolecta*.
4. **CITATION:** Ward, G.S. 1993. Oxine Copper (Copper 8-Quinolinolate): Acute Toxicity to the Saltwater Green Alga, *Dunaliella tertiolecta*, Under Static Test Conditions. Laboratory Project ID No. J9006014L. Conducted by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 429024-05.
5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *D. tertiolecta* exposed to oxine copper were 9.0, 14.3, and 15.4 $\mu\text{g ai}/\text{l}$, respectively.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: The *Dunaliella tertiolecta* culture used in the test came from laboratory stock cultures originally obtained from the University of Texas, Austin. Stock cultures were maintained in saltwater algal medium under 4.2 klux continuous illumination, at a temperature of $24 \pm 2^\circ\text{C}$. The culture used as inoculum in this test was seven days old.

B. Test System: Test vessels used were sterile 250-ml glass flasks which were capped. The test medium was the same as that used for culturing with the pH adjusted to 8.0 and filter sterilized ($0.45 \mu\text{m}$). The salinity of the medium was 30 parts per thousand (ppt).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept in a growth chamber which illuminated the vessels with fluorescent lighting continuously at an intensity of 4.0-5.2 klux. The temperature was maintained at $24 \pm 2^\circ\text{C}$.

A stock solution containing 0.7% radiolabeled material and 99.3% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine copper in the stock solution was 1 mg active ingredient (ai)/ml. Appropriate amounts of stock solution were added to nutrient solution (500 ml) to create the treatment solutions.

C. Dosage: Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 8.19, 13.3, 22.1, 36.9, 61.4, and 102 $\mu\text{g ai/l}$ were selected for the definitive test. A solvent (0.1 ml DMF/l) and a medium control were also prepared.

D. Test Design: Each treatment and control was replicated three times. An inoculum of *D. tertiolecta* cells was added to each flask that resulted in a cellular density of 3,000 cells/ml. The inoculum volume was 0.3 ml per flask. The flasks were randomly positioned in the chamber on a shaking table (100 rpm). Cellular counts were performed daily by direct microscopic examination using a hemacytometer. After 5 days of exposure, composite samples of the three replicates of the three highest concentration treatment solutions and of the

control were added to fresh media and incubated for 9 days to determine whether the effects of the test material were algistatic or algicidal. The cells were enumerated on days 0, 2, 5, and 9.

The pH was measured at test initiation and termination in all control and treatment solutions. The temperature in the chamber was recorded continually and the temperature in an uninoculated flask was measured daily. Light intensity was also measured daily.

Samples were taken at test initiation and termination for analysis of the test material by liquid scintillation counting.

- E. **Statistics:** All calculations were based on mean measured concentrations. Comparisons and percent effect values were determined against the solvent control. The 5-day EC values and associated 95% confidence intervals (C.I.) were computed using probit analysis. The no-observed-effect concentration (NOEC) was determined using analysis of variance and Dunnett's test ($p \leq 0.05$).

12. **REPORTED RESULTS:** Mean measured concentrations ranged from 88 to 110% of nominal (Table 1, attached). The mean measured concentrations were 9.04, 14.3, 21.0, 34.9, 56.3, and 89.8 $\mu\text{g ai/l}$.

Cell counts and percentage reduction in comparison to the solvent control for each concentration are presented in Table 2 (attached). Percent inhibition increased with increasing toxicant concentration and ranged from 7 to 100% inhibition.

Resuspension of the three highest concentration-exposed cells and control cells for nine days indicated that the test material was algistatic at concentrations of 34.9, 56.3, and 89.8 $\mu\text{g ai/l}$.

Based on day-5 cell counts, the EC_{50} was determined to be 15.4 $\mu\text{g ai/l}$ with a 95% C.I. of 14.5-16.2 $\mu\text{g ai/l}$. The NOEC was determined to be 9.04 $\mu\text{g ai/l}$.

The pH was 8.0-8.1 in all treatment and control solutions at test initiation and ranged from 7.9 to 9.4 at test termination. Temperature ranged from 22.7 to 24.0°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure: The test procedures were generally in accordance with the SEP and Subdivision J guidelines; the following is a deviation:

The type of lighting was not specified. Cool-white lighting is recommended for all algal tests.

The results of the continuous temperature measurements were not reported.

- B. Statistical Analysis: The reviewer used EPA's Toxanal program to verify the EC value and Williams' test to verify the NOEC as well as determine the lowest-observed-effect concentration (LOEC). The reviewer obtained similar or the same results (see attached printouts).
- C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC₅₀ for *D. tertiolecta* exposed to oxine copper were 9.0, 14.3, and 15.4 µg ai/l, respectively.
- D. Adequacy of the Study:
- (1) Classification: Core.
 - (2) Rationale: N/A.
 - (3) Repairability: N/A.
15. COMPLETION OF ONE-LINER: Yes, 11-15-93.

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Pages 5 through 6 are not included in this copy.

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MOSSLER OXINE COPPER DUNALIELLA TERTIOLECTA 11-15-93

CONC. NUMBER NUMBER PERCENT BINOMIAL
 EXPOSED DEAD DEAD PROB. (PERCENT)
34.9 100 100 100 0
21 100 85 85 0
14.3 100 36 36 0
9 100 7 7 0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 15.85344

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN G LC50 95 PERCENT CONFIDENCE LIMITS
3 1.232816E-02 15.34686 14.45499 16.24138

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
4 2.860312E-02 1 .2368335

SLOPE = 7.191992
95 PERCENT CONFIDENCE LIMITS = 5.975649 AND 8.408334

LC50 = 15.33381
95 PERCENT CONFIDENCE LIMITS = 14.50523 AND 16.19896

LC10 = 10.21092
95 PERCENT CONFIDENCE LIMITS = 9.205847 AND 11.05041

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Dunaliella cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	sol cont	3	68.733	68.733	68.733
2	cont	3	58.467	58.467	61.167
3	9.0	3	63.867	63.867	61.167
4	14.3	3	44.067	44.067	44.067
5	21.0	3	10.333	10.333	10.333

Dunaliella cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
sol cont	68.733				
cont	61.167	1.532		1.81	k= 1, v=10
9.0	61.167	1.532		1.91	k= 2, v=10
14.3	44.067	4.995	*	1.94	k= 3, v=10
21.0	10.333	11.826	*	1.96	k= 4, v=10

s = 6.048

Note: df used for table values are approximate when v > 20.

NOEC = 9.0 µg ai/l

LOEC = 14.3 µg ai/l

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